STUDIES ON THE LIFE CYCLES OF TWO SPECIES OF FRESH-WATER MUSSELS BELONGING TO THE GENUS *ANODONTA*.[1]

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Following the pioneer work of Lefevre and Curtis (1911) in this country, much interest has centered around the investigation of the developmental cycles of the fresh-water mussels. Numerous studies have demonstrated that all members of the family Unionidæ undergo sexual development through a larval stage known as the glochidium. While Surber (1912) and others have called attention to the differences in the glochidia of many species, there are as yet many untouched possibilities of differentiating easily confused species and varieties on the basis of glochidial characters. Until quite recently it has been held that in all but two of the numerous species within this family, the glochidium must pass through a period of parasitic existence on an aquatic vertebrate before transformation to the juvenile mussel is possible. With few exceptions, fishes serve as the hosts essential for the parasitic glochidia.

It was in 1866 that Leydig made the discovery that the glochidium, after leaving the parent, completed its development as a parasite on a fish. Following that disclosure, it was believed that the parasitic life was a necessary phase in the life cycle of any fresh-water mussel. Lefevre and Curtis (1911) observed a species, *Strophitus edentulus*, which undergoes metamorphosis without parasitism, the first case cited of such a condition among the Unionidæ. This report marked an important advance in the history of the knowledge of these forms.

Three years after the discovery by Lefevre and Curtis that *Strophitus edentulus* passes through its metamorphosis in the entire absence of parasitism, Howard (1914) reported a second

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case of such development. He declared (1914: 353): "One of the mussels for which I found no natural infection and for which none have been reported was *Anodonta imbecillis* (Say)." Howard stated further (p. 354): "I have tested the reaction of the glochidia in the presence of fish and obtained evidence that they do not respond as other known parasitic forms."

As a result of further investigations, Howard (1911: 355) presented the account of obtaining both infection and encystment of *Anodonta imbecillis* glochidia. He also reported having obtained infection and encystment of *Strophitus edentulus*, and for this species in addition, declared actual metamorphosis to occur on fish. He concluded, therefore, that *Strophitus edentulus* glochidia may be facultatively parasitic, while for *Anodonta imbecillis*, "there is at least a persistence of the parasitic reaction when the glochidia are artificially brought in contact with fish."

As a result of investigations carried on at the University of Illinois, some observations have been made on the life histories of two species of fresh-water mussels belonging to the genus Anodonta, *Anodonta imbecillis*[1] and *Anodonta grandis*. Attention has been directed especially to a study of the glochidia and to their metamorphoses. In this connection, the occurrence of metamorphosis without parasitism has been restudied in *Anodonta imbecillis* and observations have also been made of both glochidia and juvenile mussels of *Anodonta imbecillis* and *Anodonta grandis*.

This work has been carried on under the supervision of Professor H. J. Van Cleave at the University of Illinois. The writer wishes to express to Professor Van Cleave appreciation for suggestions and guidance in this study. Further indebtedness is hereby acknowledged to R. E. Richardson for the identification of fishes used, and to F. C. Baker for aid in ascertaining the species of mussels concerned.

[1] Baker (1927*b*) created a new genus, *Utterbackia*, with *A. imbecillis* as type. Evidences presented in this paper eliminate one of the principle characters for differentiating this genus from *Anodonta*. Under these circumstances it seems desirable to use the older name in this publication until the exact status of *Utterbackia* is determined.

Metamorphosis without Parasitism.

Development of Strophitus edentulus.—When Lefevre and Curtis (1911) recorded their observations on the direct development of *Strophitus edentulus*, they stated that the young mussels had developed inside cords within the marsupia, reaching the stage "attained by any other unionid at the time of liberation from the host fish." The young at this stage were liberated from the marsupia by the disintegration of the cords and showed "characteristic features resembling those of *Anodonta* at the close of the parasitic period." The authors reported that they were unable to bring about the attachment of these young to fish. Since the juveniles crept about on the bottom of the dish after the manner of the young of other species in post-parasitic life, Lefevre and Curtis concluded that no subsequent parasitism was possible.

Case of Anodonta imbecillis.—Howard's discovery of *Anodonta imbecillis* passing through its metamorphosis in the absence of parasitism, brought to light a second case of this newly disclosed means of development. He secured several specimens of *Anodonta imbecillis* the latter part of November (1913). Since the species is hermaphroditic (Sterki 1898: 87), his expectations were confirmed in that all the specimens were gravid. The marsupial contents upon examination showed what at first appeared to be glochidia to be in reality "juvenile mussels with organs developed to the stage usually seen at the end of parasitism when the young mussel escapes from its host" (1914: 353–4).

In confirmation of his conclusion as to the development of *Anodonta imbecillis*, Howard stated (1914: 354): "I have tested the reaction of the glochidia in the presence of fish and obtained strong evidence that they do not respond as other known parasitic forms." He does not record, however, the species of fish used. Mature glochidia taken in March were employed which "in an exposure to fish for an hour failed to give the usual infection. A few glochidia lodged in the mouths of the fish, but no encystment could be detected. The fish showed no response." He placed especial significance upon the fact that the glochidia of *Symphonota complanata* rapidly became attached to the fish which showed considerable uneasiness in marked contrast to the behavior of fishes in the presence of *Anodonta imbecillis* glochidia.

Upon this premise, Howard thought that he had conclusive evidence that *Anodonta imbecillis* passes through its metamorphosis in the absence of parasitism.

As a result of further investigation, Howard was forced to modify his conclusions, for he reported (1914: 355) both infection by and encystment of *Anodonta imbecillis* glochidia. Howard does not record the host used in his experiments, but, since the susceptibility of fishes is significant in this connection, it is possible that in his earlier experiments he used a species entirely immune to *Anodonta imbecillis*.

Occurrence of Parasitism in *Anodonta imbecillis*.

In the present investigations, it has been found that *Anodonta imbecillis* not only discharges glochidia but that they are capable of attachment upon a fish (*Apomotis cyanellus*). The period of parasitic life is of practically the same duration as is necessary for the metamorphosis of *Anodonta grandis*.

In the course of this investigation, constant reference has been made to a comparison between conditions in *Anodonta imbecillis* and *Anodonta grandis* for the developmental cycles of these two species has been studied from a comparative point of view.

Three individuals of *Anodonta imbecillis* obtained from Lake Decatur at Decatur, Illinois, in November, 1926, were placed in a tank of running water and kept under observation. Of these, one died December 3 and was found to contain only a few living glochidia in the outer gills. The remaining two specimene wers placed in separate containers January 27. On February 1, one of these was found to have given off living glochidia which were recovered by a pipette from the bottom of the jar. During a period of twenty-four hours following, approximately 200 glochidia were discharged, which were followed by similar numbers until February 8, when the mussel was found with gaping valves. Upon examination the marsupia showed a very few of the larvæ. In the meantime, the second specimen of *Anodonta imbecillis* (2/4/27) also gave off living glochidia. After two weeks, these ceased to be discharged, the mussel living, however, until March 17, at which time an examination of the marsupia showed a

few glochidial shells present but no living glochidia. It is possible that these glochidia were given off unnaturally as a result of changed environmental conditions. At any rate, the discharge was unquestionably of mature glochidia and not of young juvenile mussels.

A fairly large proportion of the glochidia discharged were active, opening and closing their valves in characteristic fashion, but in so far as these observations went were incapable of locomotion. A small green sunfish (*Apomotis cyanellus*) was subjected to a suspension of some 450 of the glochidia which had been discharged by the second specimen of *Anodonta imbecillis*. A count made after thirty minutes of exposure showed more than fifty of the larvæ attached in fairly equal distribution to the several fins of the fish. After twenty-one hours, the attached glochidia were surrounded by host tissue, encystment having been complete for at least part of the larvæ.

The infected fish was observed almost daily for the numbers of glochidia remaining. During the first few days a considerable dropping off of originally attached larvæ was noticeable. It is probable that individuals having obtained poor attachment were thus early lost.

On the eighteenth day after infection, two juveniles were recovered from the bottom of the battery jar containing the host fish. On the following days additional juveniles were secured. The following table records the number of attached larvæ and the duration of parasitism.

TABLE I.

RECORD OF THE NUMBERS OF INFECTIONS AND THE DURATION OF PARASITISM IN *Anodonta imbecillis*.

Apomotis cyanellus.	Feb.					March.										Duration of Infection.
	19	21	23	25	28	2	4	5	7	8	9	10	11	12	15	
Glochidia attached.	50	50	40	35	30	25	23	22	22	19	12	6	2	1	0	18–22 days
Juveniles recovered										2	4	3	1	1		

While Howard has reported *Anodonta imbecillis* to undergo metamorphosis without parasitism, the results of the present

study do not confirm his results. This divergence in results indicates the possibility of recognizing two distinct physiological varieties of *Anodonta imbecillis*. Howard does not give a description of the species, but the characters of the glochidia presented by Surber for *Anodonta imbecillis* agree in detail with those of the species here studied. Since preserved specimens which were collected at various times of the year yielded only glochidia, showing no evidences of transformation to the juvenile stage, it is necessary to conclude that the young of *Anodonta imbecillis* forming the basis of this study are discharged as glochidia. According to the present observations, the glochidia were given off under the conditions already stated, and some actually underwent metamorphosis upon the green sunfish (*Apomotis cyanellus*).

In this connection, it may be worth mentioning that Howard presumably worked with specimens from a large stream (the Mississippi River), while the individuals forming the basis of this study were all from relatively small streams and an artificial lake formed by damming a small stream. Utterback (1916: 2) and Baker (1927a: 112) have shown that stream conditions profoundly influence the features of the adult shell in Unionidæ. Later work supplementing that given in this paper may lead to the recognition of correlation between the differences in breeding habits and environmental factors.

The Life Cycle of *Anodonta grandis*.

As mentioned earlier in this paper the observations and experiments on the development of *Anodonta imbecillis* have been paralleled by a series of similar studies on another species, *Anodonta grandis*.

A number of living specimens of *Anodonta grandis* were obtained from Lake Decatur, Decatur, Illinois, in November, 1926. A few were examined at once and found to be gravid. The marsupia were greatly distended bearing enormous numbers of living glochidia. Measurements of the shells were made from living glochidia supplemented later by measurements from permanent microscopic mounts. Table II. records the measurements taken.

TABLE II.

RECORD OF MEASUREMENTS OF GLOCHIDIA OF *Anodonta grandis*.

	Place and Date of Collection.	Extremes of Measurement.		Most Frequent Size.	
		Length.	Depth.	Length.	Depth.
Specimen No. I. .	Decatur, Ill., 11/6/26	0.350 mm.–0.390 mm.	0.343 mm.–0.382 mm.	0.358 mm.	0.350 mm.
Specimen No. II. .	Decatur, Ill., 11/6/26	0.350 mm.–0.398 mm.	0.358 mm.–0.390 mm.	0.366 mm.	0.358 mm.

In the literature, there is a marked discrepancy between the measurements recorded for the glochidia of *Anodonta grandis*. Thus Surber (1912: 8) states that specimens which he studied were 0.41 mm. by 0.42 mm., while Ortmann (1919: 139) found the glochidial shells to be 0.36 by 0.37 mm. The latter author called attention to the difference between his measurements and those of Surber but offered no explanation for the disagreement.

Conchologists have long recognized a subspecies of *Anodonta grandis* characteristic of the large rivers under the name of *Anodonta grandis gigantea*. The differentiation of this variety from the typical *Anodonta grandis* has been largely upon shell characters. It is known that the material examined by Ortmann (1919: 140) was from a small stream as was also the material upon which the present investigation was based. On the other hand, it is probable that Surber's material, though recorded as *Anodonta grandis*, was from the Mississippi River and consequently belonged to the variety *Anodonta grandis gigantea*. Thus it seems reasonably certain that *Anodonta grandis* and its variety *Anodonta grandis gigantea* may be differentiated with greater certainty on the basis of glochidial measurements than on shell characters of the adult.

Under conditions of the experimentation, specimens of *Anodonta grandis* kept in tanks of running water continued to retain living glochidia within the marsupia from November 22 until January 6. Following this date, some of the females bore glochidia in the anterior region of the marsupia only, while after February 10 all the females examined lacked living glochidia. According to observations of Coker, Shira, Clark, and

Howard (1921: 142), females of *Anodonta grandis* bearing glochidia have been observed in December, January, February, and March only. Thus while there is a slight difference between the observations recorded by these authors and those of the present study, the length of the gravid period is almost identical in the two instances.

Four days after the living specimens of *Anodonta grandis* had been placed in the tank, material examined from the bottom contained living glochidia. In two weeks the number of living ones was greater in a similar quantity of material, which contained also many glochidial shells. The dates of gravidity for those specimens kept under observation in running water have already been indicated. Three living specimens brought in from the field on May 7, 1927, contained no glochidia.

In order to observe the results of artificial infection with *Anodonta grandis* for a comparison with the earlier study on *Anodonta imbecillis*, several specimens of small fishes were used. One species of minnow (*Pimephales notatus*), two of darters (*Etheostoma cæruleus*, *Beleostoma nigrum*), one sucker (*Moxostoma breviceps?*), and the green sunfish (*Apomotis cyanellus*) were placed in the tank with the living *Anodonta grandis* December 6, 1926. At this time, a fairly large number of living glochidia were present in the material at the bottom of the tank. A record was kept of the numbers of glochidial attachments for three weeks. The results are shown in the following table.

TABLE III.

RECORD OF THE ARTIFICIAL INFESTATIONS BY GLOCHIDIA OF *Anodonta grandis*.

Fish Introduced into the Tank Dec. 6, 1926.	Numbers of Infestations.					
	Dec. 8.	Dec. 13.	Dec. 16.	Dec. 18.	Dec. 23.	Jan. 3.
Pimphales notatus:						
Specimen No. I	1	1	3	2	1	5
Specimen No. II	0	1	3	2	1	2
Specimen No. III	0	3	1	1	1	0
Etheostoma cæruleus	0	2	14	17	11	0
Beleostoma nigrum	0	1	4	5	2	0
Moxostoma breviceps?						
Specimen No. I	0	0	21	37	5	2
Specimen No. II	0	0	2	2	0	1
Apomotis cyanellus	1	1	1	4	5	5

The results in the case of the minnows, darters, and sucker showed considerable variation in numbers of infestations. Increased attachments were evident in all cases after the first infection, which condition was especially marked in the case of one darter (*Etheostoma cæruleus*) and one sucker (*Moxostoma breviceps?*). Particularly in the case of the darter with its habit of remaining near the bottom of the tank darting about actively at times, it is not surprising to find that fairly heavy infection resulted. In the cases of the fish first mentioned, the increased infections were shortly followed by a dropping off of the glochidia, as indicated by the reductions of the numbers carried. Examination of the fish showed parts of the fins sloughed off presenting noticeably ragged appearances especially marked in the case of the darters and suckers. The sunfish, on the contrary, did not show such a reduction in the numbers of glochidia. The fins remained intact, and examination soon showed the glochidia encysted. The sunfish was the only host encountered in these experiments which retained the parasites for any appreciable length of time. Lefevre and Curtis (1910: 103) recorded several species of Centrarchidæ as natural hosts of *Anodonta grandis*.

In order to observe the results of attachment of the glochidia to the sunfish more accurately than was possible by leaving the fish free to swim about in the tank, two small uninfected specimens were used for experimentation. Of these, one was a small green sunfish (*Apomotis cyanellus*), the same species which had appeared earlier to be subject to infection, and the other a rock bass (*Ambloplites rupestris*). A fairly large quantity of living glochidia from one of the gravid females of *Anodonta grandis* was placed with each of these fish in separate small containers. Infection was evident at once, and after fifteen minutes of exposure, each fish was placed in fresh water, again in the small containers which were now set in the tank in order to keep the water at a temperature favorable for the existence on the fish, at the same time preventing additional infection.

The two fishes kept under observation were kept in the same temperature conditions, the glochidia which they carried undergoing metamorphoses in eighteen days and in seventeen to twenty days respectively. A record of the duration of parasitism on the different fishes is given in Table IV.

Table IV.

Record of the Numbers of Infections and Duration of Parasitism of *Anodonta grandis*.

	Feb.							March.					Duration of Infection.
	16	18	19	21	23	25	28	2	4	5	7	8	
Apomotis cyanellus:													
Glochidia attached.....	2	2	2	2	2	2	2	2	2	0			18 days
Juveniles recovered.....										2			
Ambloplites rupestris:													
Glochidia attached.....	40	40	35	30	25	20	17	6	4	1	1	0	17–20 days
Juveniles recovered.....									2		3		

Summary.

1. *Anodonta grandis* and *Anodonta imbecillis* have been studied with reference to the glochidia and later stages in the life cycles.

2. Through experimental infestation, glochidia of *Anodonta imbecillis* have been reared under laboratory conditions through the period of transformation to the juvenile stage.

3. An examination of mature individuals collected at various times of the year yielded only glochidia, showing no evidences of transformation to the juvenile stage.

4. The claim that metamorphosis in *Anodonta imbecillis* occurs in the marsupia of the parent has not been confirmed by this investigation.

5. *Apomotis cyanellus* served as host to the glochidia of *Anodonta imbecillis*.

6. The transformation of glochidia of *Anodonta grandis* has been followed in experimental infestations using *Apomotis cyanellus* and *Amploplites rupestris* as hosts.

7. *Anodonta grandis* may be differentiated from *Anodonta grandis gigantea* on the basis of glochidial measurements.

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